한국식물병리학회지 3(3): 198-202(1987)
Korean J. Plant Pathol. 3(3): 198-202(1987)

Measurement of Dilution End-Points and Phytotoxicity of Toxic Metabolites Produced by *Helminthosporium sativum* in Barley, Wheat and Lettuce Roots

Sang. S. Lee

Department of Biology Education, Korean National Univeraity of Education, Gang Lae Myun, Chong Won Kun, Chong Puk, 320-23, KOREA

*Helminthosporium sativum*가 생성하는 독소물질에 대한 Phytotoxicity 및 Dilution end-Points 측정 방법 개발

李 相 宣

韓國教員大學校, 生物教育科

ABSTRACT

Toxic metabolites ('Toxins'), produced by *Helminthosporium sativum* causing leaf blotch in barley and root rot in barley and wheat were partially purified through C-18 column. The partially purified toxins appeared heat unstable and lipophilic. The responses of toxins to wheat and barley root corresponded with those to lettuce growth with the different concentrations. The determination of the concentration of toxins produced was developed using the dilution end-points. The equation [Y = a log X + b] was obtained from the semi-log graphy with the linear analysis. The values 'a' and 'b' were discussed with the responses of several plants on the toxin produced by *H. sativum*.

Key words: Helminthosporium sativum, toxin, dilution end-point.

要 約

밀 보리에서 crown root rot 및 leaf blotch 를 이르키는 *Helminthosporium sativum* 의 毒素物質을 分離하였다. 이는 毒素物質에 대한 어떤 손상도 없이 C-18 column 으로 分離되었으며, 實驗的으로 가능한 inhibitor 를 제거하였다. Lettuce 成長과 毒素물질의 濃度의 反應에는 semi-log 표에서 비례하였다. 또한, pathogen host 인 밀 보리의 뿌리 成長에 사용한 結果 Lettuce 의 反應과 同一한 結果를 얻었다. 毒素물질과 植物의 反應을 測定하여 dilution end-points 의 결과로, 직접적으로 毒素物質의 濃度를 測定하는 方法을 改發하였다. [Y=a log x +b] 의 公式과 通系處理로 사용하여 얻은 a 와 b 의 意味를 討論하였다.

이 논문은 1987 년도 문교부 자유공모과제 학술연구조성비에 의하여 연구되었음.

INTRODUCTION

Several different terms (selective or non-selective toxin) related to the concept of toxins have been employed for different responses to toxins in the host plants (3, 5, 12, 14). Scheffer (13, 14) and Daly (2, 3) used the terms, "host specific toxin", whereas wheeler (16) employed the term, "host selective toxin". However, their terms expressed for *Helminthosporium* toxins seemed to be applicable to the same interaction between *Helminthosporium* species and their host plants, but not for diseases caused by the pathogen.

Considerable discussion of the terminology of plant toxins have continued (3, 12, 13, 14), but the practical aspects for plant toxins have rarely been discussed. Wheeler (16) employed the term, "dilution end-point" for the expression of toxin concentration or plant toxicity (host selective toxin) in *Helminthosporium* oat blight. The method to measure the toxin concentration and phytotoxicity in the interaction have not been clearly discussed.

Our objective was to estimate the dilution endpoints as accurately as possible and phytotoxicity of *H. sativum* toxic metabolites (referred "toxins") for the host crop species. The toxins referred here and obtained from *H. sativum* was not defined as host-selective toxins.

MATERIALS AND METHODS

H. sativum P. K. & B. isolate R002 was obtained from the common root rots in diseased plants in the North Dakota wheat fields, and its pathogenicity evaluated under standard conditions (4, 6). The toxins was prepared from shake cultures as the previously described (8). Lettuce seeds (*Lactuca sativa* L. light sensitive, Carolina Biological Supply Co., Burlington, NC) were surface disinfested in 30% H_2O_2 for 2 min, followed by 2 or 3 rinses of sterile distilled water. Seeds were placed on the water agar in petri dishes at 22-25C under the fluorescent lights for 24 hrs to germinate. For each bioassay plate, ten lettuce seedlings of uniform length (4 or 5 mm) were selected and placed on two percent water agar in petri dishes and floodes with two ml of culture filtrate. After three days' incubation at 25 C, the root lengths of them were measured.

The dilutions of culture filtrate with the sterilized culture broth or the dilution of C-18 column prepared toxins with the distilled water was conducted for evaluation of toxicity. Wheat (*Triticum aestivum* L. cv. Thatcher and Calvin) and barley (*Hordeum vulgare* L. cv. Larker and Dickson) lines were employed for this experiment (4). All seeds were surface-sterilized and washed by the lettuce method previously described. Seeds (barley or wheat) having 6-10mm roots were carefully placed on the test tube under the microhood. Roots were carefully examined under the microscope for symptoms. These symptoms shown in Figure 1 were consistent with others (1, 4, 9). All experiments shown in this paper were done, as based on the root growths resulted from these symptoms shown in Figure 1.

The culture filtrate was extracted using soild phase extraction column (1 ml) composed of octadecyl silane bonded to silica (Baker disposable extraction column 7020-1, C-18). The detailed procedures for toxin extraction was described in the

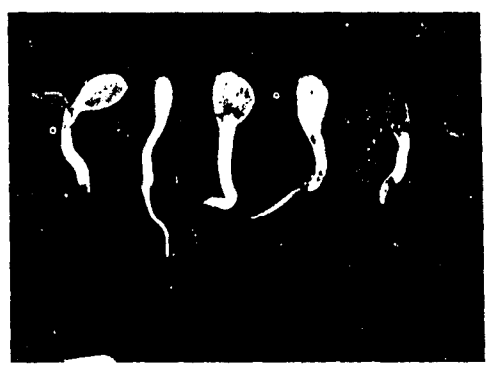

Fig. 1. The lettuce seedlings grown at 25 C for 3 days in toxin solution and the dark areas in roots indicating the damages from *H. sativum* producing toxins.

manual of commercial products. Twenty to 25 ml of culture filtrate was passed through the C-18 column, collected, dried at 40 C under mild vaccum and compared with the original culture filtrate for activity in the plant root responses. The toxins were disolved in methanol, diluted with the distilled water to reach a concentration of 0.5%, and used in plant bioassays. Lettuce root growth responses were plotted at the 2,560 dilution rates (15).

RESULTS

Root growth responses (inhibition) in lettuce was linear with the log of the toxin concentration after serial dilution to 1:2,560 (R^2= 0.99 and 0.98 for the culture filtrate and C-18 purified toxin, respectively). The toxin response rate (the slope of purified or culture filtrate toxins vs. the dilution) was different from each other. Figure 2 shows the lettuce root response to *H. sativum* toxin before and after passing the culture filtrate through the C-18 column. The C-18 column retained 97% of toxin activity based on dilution end-points (1:1,568 or 1:1,522 for culture filtrate or purified toxin, respectively). The toxicity of the culture filtrate disappeared after autoclaving it at 126 C for 10 min.

Root growth responses of barley and wheat seedlings to different concentrations of C-18 column-purified toxins were measured (Table 1). The effects of the purified toxin on different species were similar. All showed the log-linear response to concentration. The R^2 values on log-linear regressions were highly significant. The toxins produced by *H. sativum* killed the cells located at the meristematic tissues in the roots of three crop species and stimulated the development of the lateral (branched) roots in lettuce. In the 3 days of observation, the lateral roots were also killed. The toxin affected root growth only in the three different plant species, but did not kill coleoptiles. The crop root growth of Dickson or Thatcher, known to be the resistant, was more sensitive to toxin concentration than was Larker or Calvin, but the toxicity of two barley lines appeared different from each other. These results indicated that the resistant lines showed higher values in dilution end-points, whereas they showed lower values in phytotoxiity (Table 1).

DISCUSSION

The linear responses for log of toxin concentrations in lettuce were consistent with the results of Davis and Christ (1, 4). Figure 2 indicated that toxin(s) produced by *H. sativum* was recovered at a high rate (97%) by the C-18 column. The results

Table 1. Wheat, lettuce and barley responses for C-18 column prepared *Helminthosporium sativum* toxin[a]

	Plant root length responses at 25 C for 3 days[b]				
	Y = a (Log X) + b				Dilution
Crops	a	b	R^2 (df)	Toxicity, %[c]	end-point[d]
Lettuce	.322	.880	.98 (5)	72.8	1:1522
Wheats Thatcher	.861	1.870	.98 (3)	71.1	1:206.6
Calvin	.981	1.750	.98 (3)	72.1	1:172.3
Barleys Larker	.869	1.207	.99 (3)	73.5	1:48.6
Dickson	.785	1.568	.96 (3)	66.8	1:56.1

[a]The *C. sativus* toxin was prepared from C-18 column extraction and applied to the plant as the 2 ml culture filtrate equivalent.

[b]The units of Y, a and b in the equation were cm, per cm and cm, respectively.

[c]Toxicity, percentage, was calculated as the value of 100 X (root length in the water (rlw) - root length in dilution 1) / rlw.

[d]Dilution end-point was the X values when Y = root length in the the water (rlw).

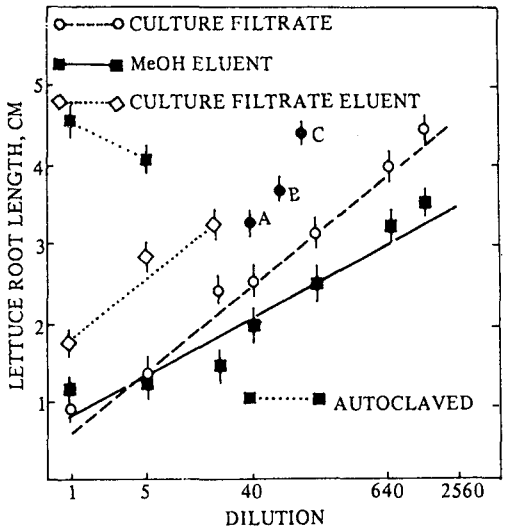

Fig. 2. The dilution end-points and pathotoxicities of toxic metabolies treated. The dilutions was made with sterile distilled water or culture broth. Culture filterate eluent, Y = .502 Log (Dilution = X) + 1.832 (R^2 = 0.97, df = 1). MeOH eluent (C-18 treatment), Y = .322 Log X + 0.880 (R^2 = 0.98, df = 5). Original culture filtrate, Y = .495 Log X + 0.798 (R^2 = 0.99, df = 5). A= MeOH control. B = Water control. C = Sterile culture broth.

of the purified toxins shown in Table 1 were similar to those experiments on wheat seedlings using culture filtrates (4). Harding (7) and Ludwig (9) measured the total seedling length (root plus coleoptile) of wheat and barley when they were immersed in culture filtrates. Their results were also supported by our results in root length responses in wheat and barley. The lettuce growth technique is consistent with the results of barley and wheat and is simple and easy to use when *H. sativum* toxins was applied.

Since the C-18 column retained only hydrophobic compounds, it eliminated other possible plant inhibiting compounds such as asparagine (4) or organic acids (10, 11). Preliminary studies suggested that the toxin produced by *H. sativum* was a hydrophobic and heat unstable compound.

Several terms should be defined: phytotoxicity, dilution end-points, and phytotoxin response, to understand the phytotoxin behavior because the plant responses for toxins are not linearly related to the concentration of phytotoxin. The values determined in the early works were confusing and difficult to compare with other data. The determination of these values was not clear and inadequate in the experimental methods.

The phytotoxicity should be related to the plant response at the given toxin concentration, but it is not related to the concentration of the toxin. So, it should be employed for comparison of plants or different lines of plants at the given concentration. The dilution end-points should be related to the toxin concentration or amounts at the given test plants, and it is also related to phytotoxicity. So, the dilution end-points should be applied to the comparison of toxin production by different fungal isolates within a species. The following mathematic equation was derived from Figure 2:

$$Y = a \text{ Log } X + b$$

Y is the plant response at the given dilution rate, X. "a" and "b" values should be calculated from the statistical analysis. The dilution end-point, X_{DEX}, would be calculated from the control root response, Y_{DEP}, without any detailed dilution experiments. Also, phytotoxicity was calculated when X = 1. The two values of culture filtrate and purified toxin of *H. sativum* were not real values at the original culture and purified toxin because the toxin at the original solution was too toxic to measure. So, Yo (= b), the plant reponse in the original toxin solution (when x = 1), was accurately estimated from the statistical analysis. Phytotoxicity (Pt) should be defined as following:

$$Pt = (Y_{DEP} - Yo) / Yo \quad \text{or}$$

$$Pt = (a / Y_{DEP}) \text{ Log } (X_{DEP} / Xo)$$

Here, X_{DEP} and Pt should be a variable of the plants, such as lettuce, two malting barleys, and wheat. In other words, the phytotoxin response, a/Y_{DEP}, should be specific for the interaction between the plant and the toxin, and should be defined. Therefore, X_{DEP} value have often been employed for the specific phytotoxin in disease

caused *H. victoria*

REFERENCES

1. CHIST, J. E. (1979). Growth and toxin production of cultured *Helminthosporium sativum* Pam. King & Bakke. M. S. thesis, Univ. Missouri, Columbia 88p.
2. DALY, J. M., & KNOCHE, H. W. (1976). Hormonal involvement in metabolism of host parasitic interactions. page 117-133. In *Biochemical Aspects of Plant-Parasite relationships.* Friends, A. S. & T. L. Trelfall, eds. Academic Press, London 850p.
3. DALY, J. M., & KNOCHE, H. W. (983). The chemistry and biology of pathotoxins exhibiting host selectivity. page 83-138. In *Advances in Plant Pathology.* Vol I. D. S. Ingram & P. H. Williams, eds. Academic Press, London and N. Y. 367p.
4. DAVIS, C. M. (1983). *Helminthosporium sativum* P. K. & B. toxic metabolites: wheat cultivar response and lettuce bioassay. M. S. Thesis, North Dakota State Univ., Fargo, 86p.
5. DIMOND, A. E., & WAGGONER, P. E. (1953). On the nature *Phytopathology* 43: 229-235.
6. EL-NAAHAAR, H. (1984). Pathogenic variations, host specialization and aggressiveness in *Helminthosporium sativum.* Ph. D. Thesis, North Dakota State Univ., Fargo. 91p.
7. HARDING, H. (1971). Effect of *Biopolaris sorokiniana* on germination and seedling survival of varieties or lines of 14 *Triticum* species. *Can. J. Bot.* 49: 281-287.
8. LEE, S. S. (1986). Toxic metabolites produced by *Helminthosporium sativum.* Ph. D. Thesis north Dakota State Univ., Fargo, ND. 120p.
9. LUDWIG, R. A., CLARK, R. A., JULIN, J. B., & ROBINSON, D. B. (1965). Studies on seedling diseases of barley caused by *Helminthosporium sativum* P. K. & B. *Can. J. Bot.* 34: 653-673.
10. PRINGLE, R. B. (1976). Role of toxins in etiology of root rot disease of wheat. *Can. J. Bot.* 55: 1801-1806.
11. PRINGLE, R. B. (1979). Role of toxins etiology of spot blotch disease of barley. *Can. Plant Dis. Sury.* 59: 74-79.
12. PRINGLE, R. B., & SCHEFFER, R. P. (1965). Host-specific plant toxins. *Ann. Rev. Plant Pathol.* 2: 133-156.
13. SCHEFFER, R. P. (1983). Toxins as chemical determinations of plant disease. page 1-40 In *Toxin and Plant Pathogenesis* Daly, J. M., & B. J. Deverall. eds. Academic Press, London. 485p.
14. SCHEFFER, R. P., & YODER, D. C. (1972). Host specific toxin and selective toxicity. page 251-268 In *Phytotoxins in Plant diseases* R. K. Wood, A. Ballio, & A. Graniti, eds. Academic Press, London. 418p.
15. WHARTON, D. C., & McCARTY, R. E. (1972). *Experiments and Methods in Biochemistry.* The McMillan Co. New York. 350p.
16. WHEELER, H. (1975). *Plant Pathogenesis.* See page 24-42. Springer-Verlag, Heidelberg-Berlin. 150 p. illustr.